

A validation study of after reconstitution stability of diabetes: level 1 and diabetes level 2 controls

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Key words:

HbA1c, six sigma, decision charts,
total allowable error, HPLC

Limitation of the study:

Involvement of more participant laboratories could produce better scope of assessment of after reconstitution stability. But laboratories using same commercial lot was not available except one laboratory, the data from which has been supplemented.

Ethical issues:

The laboratories involved in the study used controls exclusively for validation study. For regular use to validate patient results the manufacturer's instruction has been followed.

ABSTRACT

Objective

The after reconstitution stability of L1 & L2 is 7 days when stored at 2°-8°C and tightly capped. The total content of the vial is 500 µL and per test requirement is 5 µL. Hence, in 7 days laboratories would consume only 35 µL wasting 365 µL which is 73% of the expensive control samples and such wastage should be ideally prevented. The study of after reconstitution stability proved the stability of the control samples up to 90 days resulting in proper utilization of L1 & L2.

Materials and methods

The L1 & L2 controls were reconstituted using 500 µL deionized water. The vials were allowed to stand for 5-10 minutes, swirled 8-10 times to maintain the homogeneity. Aliquots of 10 µL were prepared. One aliquot of one control level being used per day, both levels on alternate day. 5 µL control is mixed with 1.5 mL diluent and HbA1c was tested in D10 system by HPLC method. Therefore, 500µL may be utilized up to 45 days approximately provided the extended use of control run is not affecting quality of test results. The minimum number of samples tested by the laboratory from a single vial is 45 and maximum 46.

Results

Three lots were tested of which one lot has been tested in two laboratories. Mean, SD, CV%, TAE, %Bias, z-score and sigma calculations were done. The medical method decision charts were created for all lots based upon normalized operational specifications which showed excellent precision in both control levels. Number of rejections in the study was nil.

Conclusion

The extended use of controls is validated.



Abbreviations (in alphabetical order)

L1: Lyphochek Diabetic control level 1

L2: Lyphochek Diabetic control level 2

HbA1c: Glycosylated Hemoglobin

HPLC: High Performance Liquid Chromatography

TAE: Total Allowable Error



INTRODUCTION

The IUPAC technical committee recommended validation study in a single laboratory provided the study is appropriate to the fitness of purpose and the validated document is continually verified (1). The recommendation suggested to estimate measurement trueness, recovery and linearity. In the present study, the validation of stability of reconstituted analyte was in question. So, the number of rejections, CV% as an indicator of measurement trueness, %bias and z-score are the parameters to be estimated (2). As the values of the analyte have been provided in the manufacturers insert so TAE may be obtained from the same. In 1974, Westgard, Carey and Wold introduced the concept of TAE to provide a quantitative approach for judging the acceptability of method performance (2)

and on 2009, Clinical and Laboratory Standards Institute (CLSI) published the TAE of all clinical chemistry analytes (3). It has been recommended to use the CLSI table for TEA or to use 95% confidence interval of the limit of possible analytic error whichever is appropriate/less (4). If the result of reconstituted sample remains within the range provided by manufacturer apparently it may be said that the after reconstitution period may be extended. To validate the extended stability after reconstitution, Sigma should to be calculated and approved finally from medical method decision chart (5, 6). The medical method decision chart is based on sigma calculations and operating point ≥ 5 sigma is the marker of excellence of performance.

MATERIALS AND METHODS

Lyphochek Bilevel Diabetes Controls (L1 &L2) were reconstituted using 0.5 ml deionized water. Calibrated auto pipette was used for reconstitution. The stopper was replaced and the control samples were allowed to stand for 5-10 minutes. The vials were gently swirled 8-10 times before preservation in aliquots to maintain homogeneity. 10 μ L reconstituted controls were kept in each aliquot. The aliquots were preserved at 2°C-8°C (7). One aliquot was taken out every day and 5 μ L of reconstituted sample was mixed in 1.5 mL diluent. The aliquot was brought to room temperature and swirled before mixing with diluent. The L1 & L2 aliquots were used on alternate day. Hence approximately 45 aliquots of one level control would be consumed in 90 days. Three vials of Lot numbers 33870, 33890 and 33920 were tested for 90 days generating 135 numbers L1 & L2 data i.e., 135 number results generated per level per lot. Lot 33920 have been tested in two different laboratories (incidentally common lot was supplied) hence 135 number data of both levels could have been accumulated from two laboratories. Both level control samples of same lot

were reconstituted and aliquoted on the same day and time. HbA1c was measured in BIORAD D10 system by Ion Exchange HPLC method. As the objective was to establish after reconstitution stability of control samples hence method and mode needed to be user specific and widely accepted.

STATISTICAL CALCULATIONS

Statistical calculations were as per the guideline of Westgard et al (4). Mean, SD, CV%, %Bias of every lot have been calculated. Bias is the difference of laboratory mean and Peer group mean. The manufacturer's mean of Lot 33870 were 5.4% (L1), 9.9 % (L2). The peer mean were 5.38% and 9.79%. The manufacturer's mean for lot 33890 were 5.3% and 9.8%. Peer mean were 5.48 & 9.79. The manufacturer's mean for 33920 were 5.4 & 9.8 and peer mean were 5.42% and 9.84% respectively. Peer means were used for statistical calculations as per CLSI guideline and Westgard's rule. TAE is the percentage of total allowable deviation range. The ratio of manufacturer's allowable deviation

and manufacturer's target mean is expressed in % (8). When TAE is more than 20% as per CLSI guideline 20% has been considered as optimum TAE (4). From TAE, %Bias and CV% Sigma is calculated ($TAE - \%Bias / CV\%$). Method decision charts were plotted on the basis of calculations from Westgard Website.

RESULTS AND DISCUSSION

The after reconstitution stability was tested in three consecutive lots 33870, 33890 & 33920. The bias was calculated considering peer mean as target mean. As per Multi QC rule $\pm 3SD$ would be considered as acceptable allowable error (TAE) keeping the option of one day 2-3SD result in the warning range when the laboratory uses its own laboratory mean [8]. In lot 33870 calculated TAE (from peer mean and SD) of L1 was 20.9 and in 33920 both level values were 28.5 and 20. But as per CLSI guideline optimum TAE should be 20% [Table 1]. So, instead 20.9 & 28.5 the TAE was considered to be 20. No result was found to have exceeded $\pm 2SD$. Hence number of rejection/warning range result in three

Table 1 HbA1c Peer results of lots 33870, 33890, 33920

Control	Lot number	Target (%)		Peer SD		Range ($\pm 3SDX2$)		TAE (%) = Range x 100/ Peer mean	
		Peer	Insert	Peer	Insert	Peer	Insert	Peer	Insert
L1	33870	5.38	5.4	0.184	0.2	1.104	1.2	20.0	22.22
L2	33870	9.79	9.9	0.257	0.4	1.542	2.4	15.6	24.24
L1	33890	5.48	5.3	0.166	0.2	0.996	1.2	18.2	22.64
L2	33890	9.87	9.8	0.245	0.4	1.47	2.4	14.9	24.5
L1	33920	5.42	5.4	0.184	0.2	1.104	1.2	20.0	22.22
L2	33920	9.84	9.8	0.328	0.4	1.968	2.4	20.0	24.5

Table 2 Statistical evaluation of HbA1c values of Lot 33870

N	Mean HbA1c (%)	SD	CV%	Deviation from peer mean	% Bias	Tests per vial	Sigma	z-score	Allowable bias*	Allowable imprecision*	Critical error*
182	5.47	0.127	2.32	0.09	1.67	45+46 +46+45	8.0	0.49	8.3	11.6	-0.641
182	9.52	0.191	2.01	-0.27	2.96	45+46 +46+45	6.3	-1.13	19	13	-1.911

*Allowable Bias- Normal Operational Specifications along Y-axis. Calculated as: %Bias*100/TAE (%)

*Allowable Imprecision- Normal Operational Specifications along X-axis. Calculated as: CV%*100/TAE (%)

N- Number of tests performed

Critical error – Calculations obtained from “Quality Control Grid Calculator”. Critical error

< ±2.0 shows excellent performance

Table 3 Statistical evaluation of Lot 33890

N	Mean HbA1c (%)	SD	CV%	Deviation from peer mean	% Bias	Tests per vial	Sigma	z-score	Allowable bias*	Allowable imprecision*	Critical error*
180	5.56	0.127	2.285	0.08	1.46	45+45 +45+45	7.3	0.48	8	12.5	-0.834
183	9.67	0.262	2.2	-0.20	2.02	46+46 +45+46	5.9	-0.82	13.6	14.7	-1.337

lots was zero. The peer mean & SD were considered for TAE calculation as they are narrower than manufacturer’s range (Insert value), based on statistical calculation of worldwide results and system and method specific.

Two lot results showed intra laboratory precision findings. After reconstitution stability of Lot 33870 & 33890 were tested using two L1 & two L2 vials for 90 & 92 days. One aliquot tested on alternated day so 45 aliquots consumed within 90 days and 46 consumed by 92 days. The sigma values were 8.0, 6.3 & 7.3, 5.9 [Tables 2 & 3].

Z-Score calculation is recommended for accuracy check of internal quality control [9] and in both lots both level z-score are within ± 2 [Tables 2 & 3].

The inter performance testing of one lot has been done in two laboratories with lot no. 33920. Both laboratory tested 3 vials of L1 & L2 for 90 days obtaining total 270 results [Tables 4 & 5]. The sigma values were above 6 and z-scores within limit. The sigma and z-score of all three lots satisfies the criteria of standard performance.

Table 4 Statistical evaluation of Lot 33920 (Laboratory 1)

N	Mean HbA1c (%)	SD	CV%	Deviation from peer mean	% Bias	Tests per vial	Sigma	z-score	Allowable bias*	Allowable imprecision*	Critical error*
135	5.49	0.17	2.5	0.07	1.3	45+45	7.5	0.27	6.5	12.5	-0.569
						+45					
135	9.97	0.203	2.04	0.13	1.32	45+45	9.2	0.39	6.6	10.2	-0.336
						+45					

Table 5 Statistical evaluation of Lot 33920 (Laboratory 2)

N	Mean HbA1c (%)	SD	CV%	Deviation from peer mean	% Bias	Tests per vial	Sigma	z-score	Allowable bias*	Allowable imprecision*	Critical error*
135	5.55	0.23	2.2	0.13	2.4	45+45	8.0	0.5	12	11	-0.923
						+45					
135	10.18	0.206	2.02	0.34	3.45	45+45	8.2	1.03	17.2	10.1	-1.37
						+45					

The use of normalized operation specification chart (NOPSPECs) is being done from the early 1990s. The chart describes the operational limits for imprecision and inaccuracy with respect to a desired level of quality assurance for the particular analyte [10]. The chart is the medical decision chart to select a QC procedure (11, 12). The idea of the medical decision chart is to express the values on the X and Y axis as % of TAE. The X-axis is plotted as imprecision_{measure} % TAE i.e., percentage of allowable imprecision and Y axis Bias_{measure} % TAE or percentage of allowable bias of the method to be validated (Charts 1-8). The charts may be downloaded from Westgards website. Sigma would be automatically calculated from the input of TAE (%), CV% & % Bias.

The medical decision chart is based on CLSI guideline by Westgard et al where maximum allowable bias is 20% and CV is 10% resulting 2 sigma. Considering allowable Bias_{measure} %TAE 20 the colour codes in the charts showed the less the imprecision (CV %) the higher is the sigma value (2-6, colour codes red –green). The method is validated if sigma is ≥4, good if sigma is ≥5, excellent when sigma is ≥ 6. The sigma operating points below 6 are beyond the level of excellence as above 6 sigma no grade has been prescribed yet. Calculations were described below Table 2 and followed for all three lots. The operational specification chart for both controls in 3 groups were evaluated (Tables 1-5, Charts 1-8). Analytical accuracy for all the assay

performance are within optimum excellence decision range (Charts 1-8). Hence, the diabetic controls may be used for 3 months instead of 7 days after reconstitution.

As per the QC calculator rule critical error up to 2 indicates excellent precision. In the present study, the critical errors of all the lots are $< \pm 2$. The stability of the reconstituted control have satisfactorily passed both operating points and critical error criteria. So, the utilization of diabetic controls up to 90 days is acceptable if reconstituted control material is kept in aliquot at 2° - 8° C and one aliquot is being used once (13, 14).

CONCLUSION

1. The use of diabetic controls of BIORAD for extended days is validated and verified. The controls may be used for approximately 90 days provided they are preserved properly.
2. The study is having an important practical application value. The laboratory has saved 5-fold expenditure i.e. 1 control in 7 days, hence 6 control vials in 45 days approximately. Such cost saving would be beneficial for the patient care services as the laboratory would be able to perform HbA1c at a patient beneficial rate. HbA1c is a prognostic marker of diabetes so the aim of the laboratories should be to offer the test at a moderate cost.
3. Any waste should be discouraged. The study showed prevention of waste of expensive controls.

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Chart 1 Method decision chart of L1, 33870

Test or Analyte	HbA1c,33870,L1	Analyst	Dr.Shyamali Pal
Methodology	Ion Exchange HPLC	Date	
Quality Requirement		Sigma Limits	
Allowable Total Error	20.0	6	3.33
Offset	0.0	5	4.00
Method Performance		4	5.00
Bias (% diff)	1.7	3	6.67
Imprecision (% CV)	2.3	2	10.00
Sigma Metric	8.0		

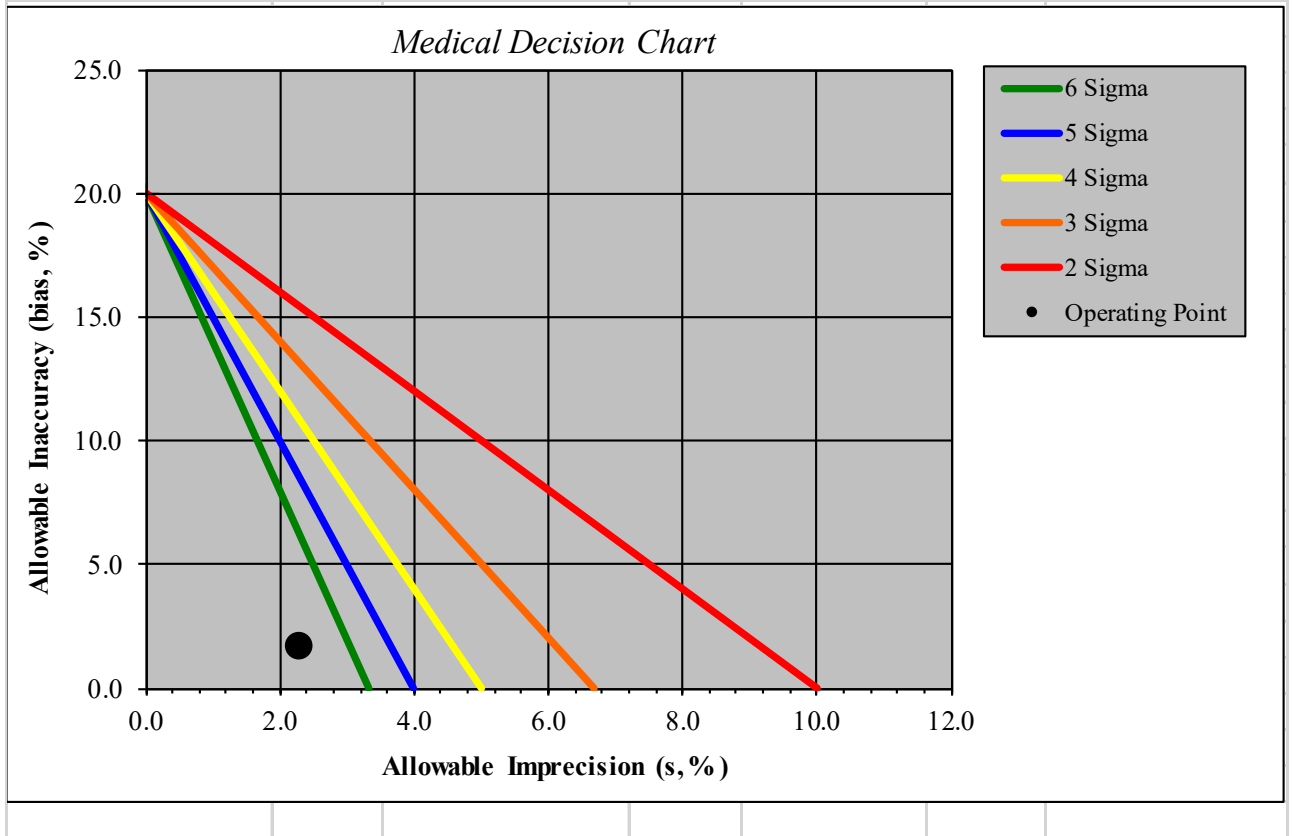


Chart 2 Method decision chart of L2, 33870

<i>Test or Analyte</i>	HbA1c,33870, L2
<i>Methodology</i>	Ion Exchange HPLC

<i>Analyst</i>	Dr.Shyamali Pal
<i>Date</i>	

<i>Quality Requirement</i>	
Allowable Total Error	15.6
Offset	0.0
<i>Method Performance</i>	
Bias (% diff)	3.0
Imprecision (% CV)	2.0
Sigma Metric	6.3

<i>Sigma Limits</i>	<i>S</i>
6	2.60
5	3.12
4	3.90
3	5.20
2	7.80

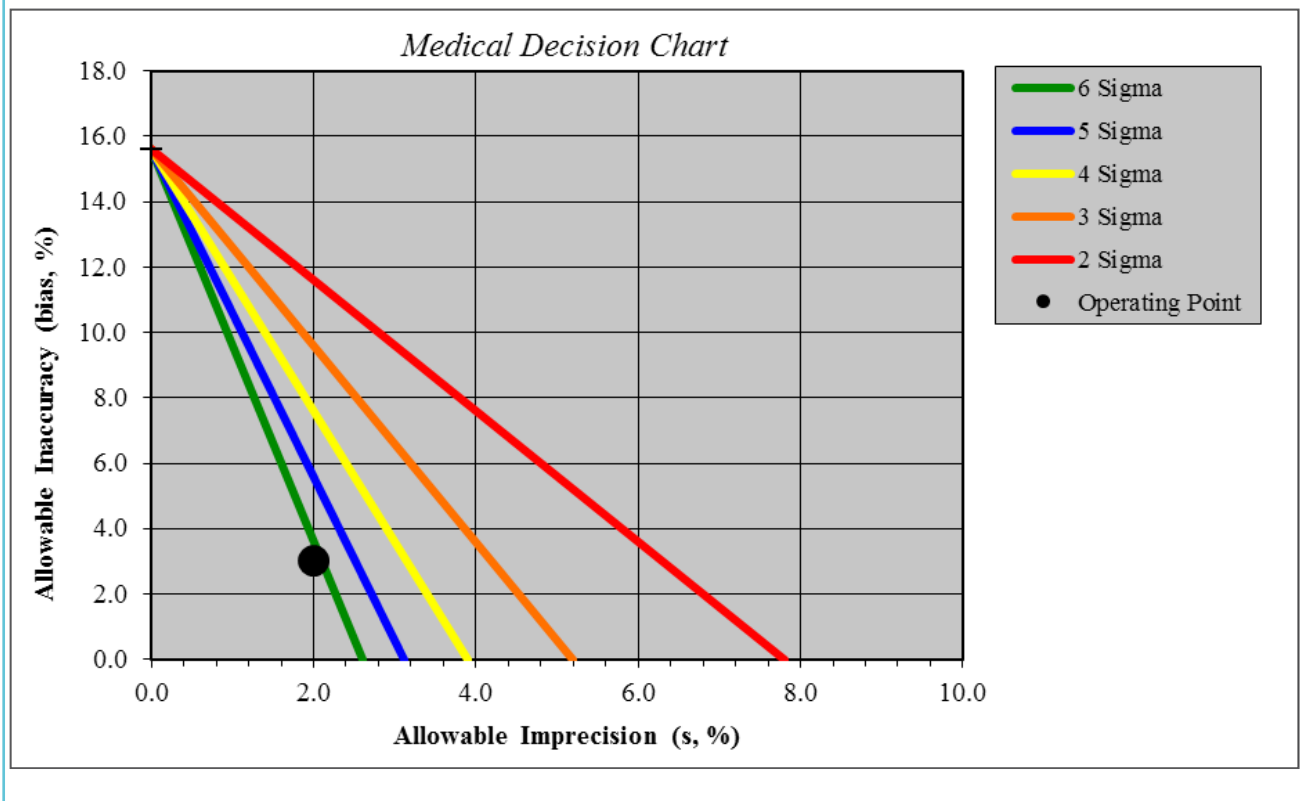


Chart 3 Method decision chart of L1, 33890

Test or Analyte	HbA1c,33890,L1	Analyst	Dr.Shyamali Pal
Methodology	Ion Exchange HPLC	Date	
Quality Requirement		Sigma Limits	
Allowable Total Error	18.2	6	3.03
Offset	0.0	5	3.64
Method Performance		4	4.55
Bias (% diff)	1.5	3	6.07
Imprecision (% CV)	2.3	2	9.10
Sigma Metric	7.3		

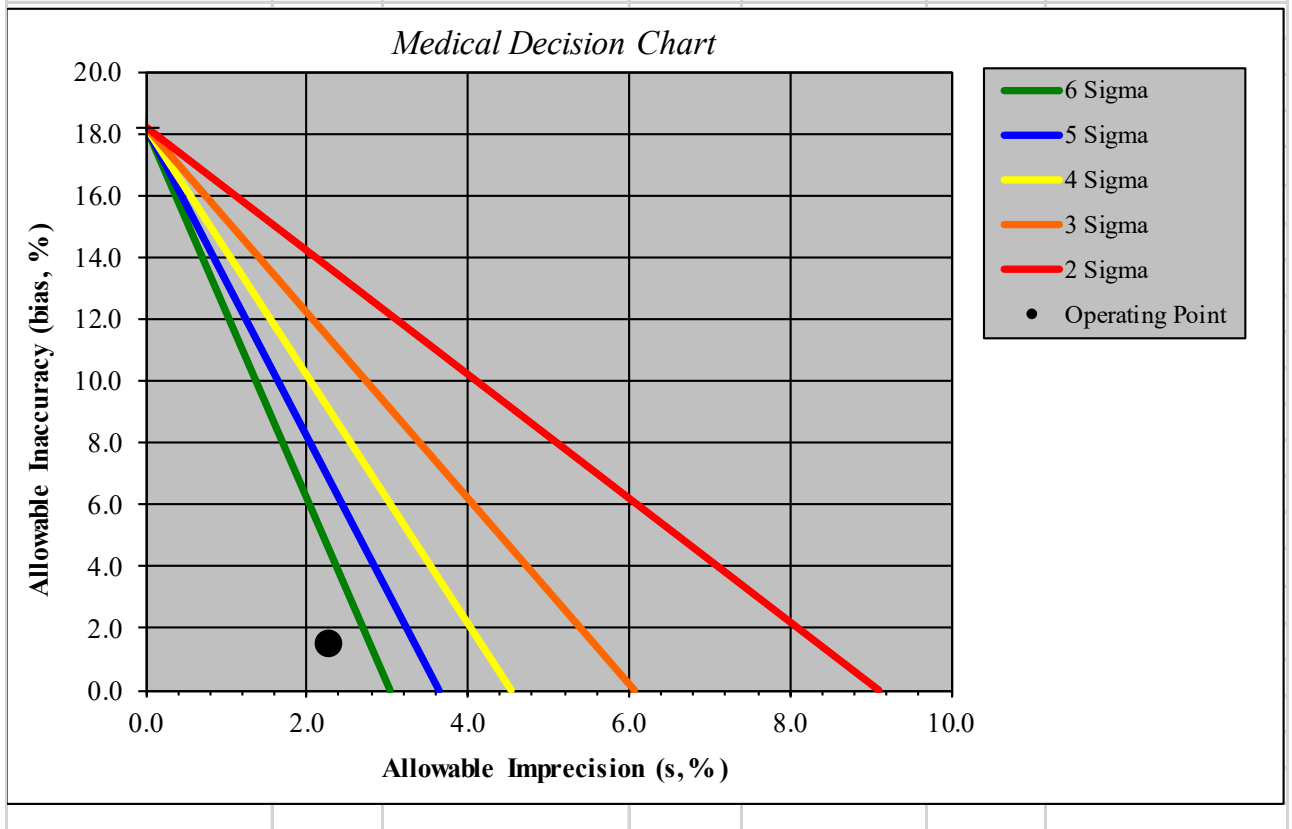


Chart 4 Method decision chart of L2, 33890

Test or Analyte	HbA1c,33890,L2	Analyst	Dr.Shyamali Pal
Methodology	Ion Exchange HPLC	Date	
Quality Requirement		Sigma Limits	
Allowable Total Error	14.9	6	2.48
Offset	0.0	5	2.98
Method Performance		4	3.73
Bias (% diff)	2.0	3	4.97
Imprecision (% CV)	2.2	2	7.45
Sigma Metric	5.9		

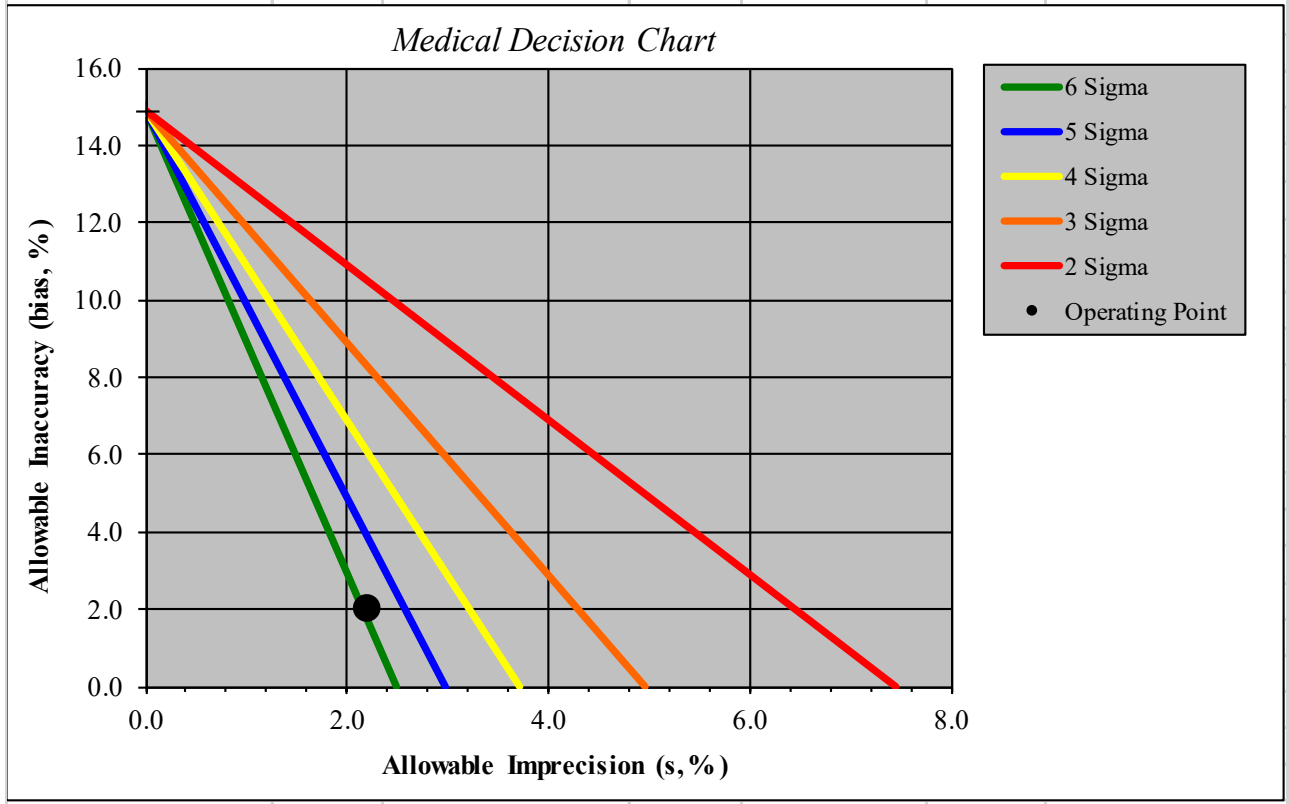


Chart 5 Method decision chart of L1, 33920 (Laboratory 1)

Test or Analyte	HbA 1c,33920,L1, Laboratory1	Analyst	Dr.Shyamali Pal
Methodology	Ion Exchange HPLC	Date	
Quality Requirement		Sigma Limits	
Allowable Total Error	20.0	6	3.33
Offset	0.0	5	4.00
Method Performance		4	5.00
Bias (% diff)	1.3	3	6.67
Imprecision (% CV)	2.5	2	10.00
Sigma Metric	7.5		

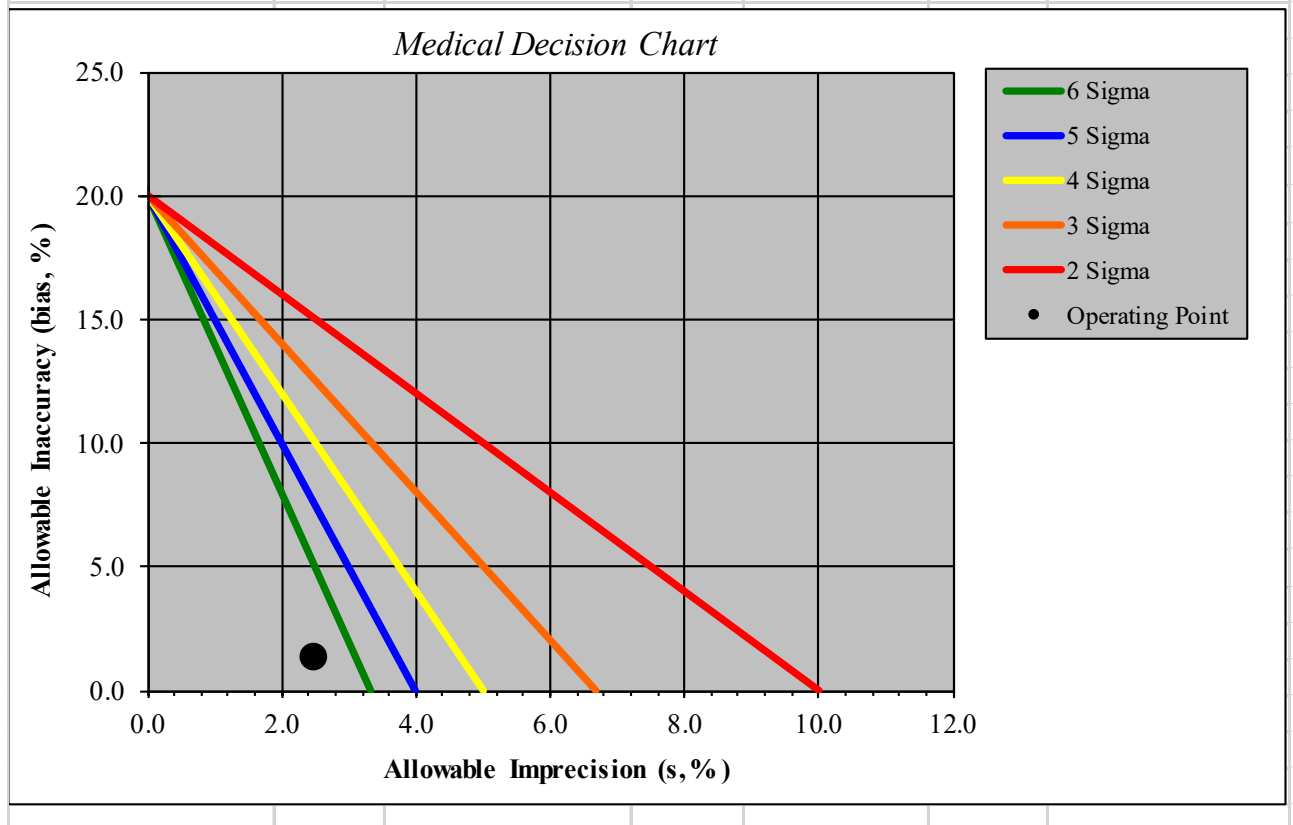


Chart 6 Method decision chart of L2, 33920 (Laboratory 1)

Test or Analyte	HbA1c,33920,L2,Laboratory 1	Analyst	Dr.Shyamali Pal
Methodology	Ion Exchange HPLC	Date	
Quality Requirement		Sigma Limits	
Allowable Total Error	20.0	6	3.33
Offset	0.0	5	4.00
Method Performance		4	5.00
Bias (% diff)	1.3	3	6.67
Imprecision (% CV)	2.0	2	10.00
Sigma Metric	9.2		

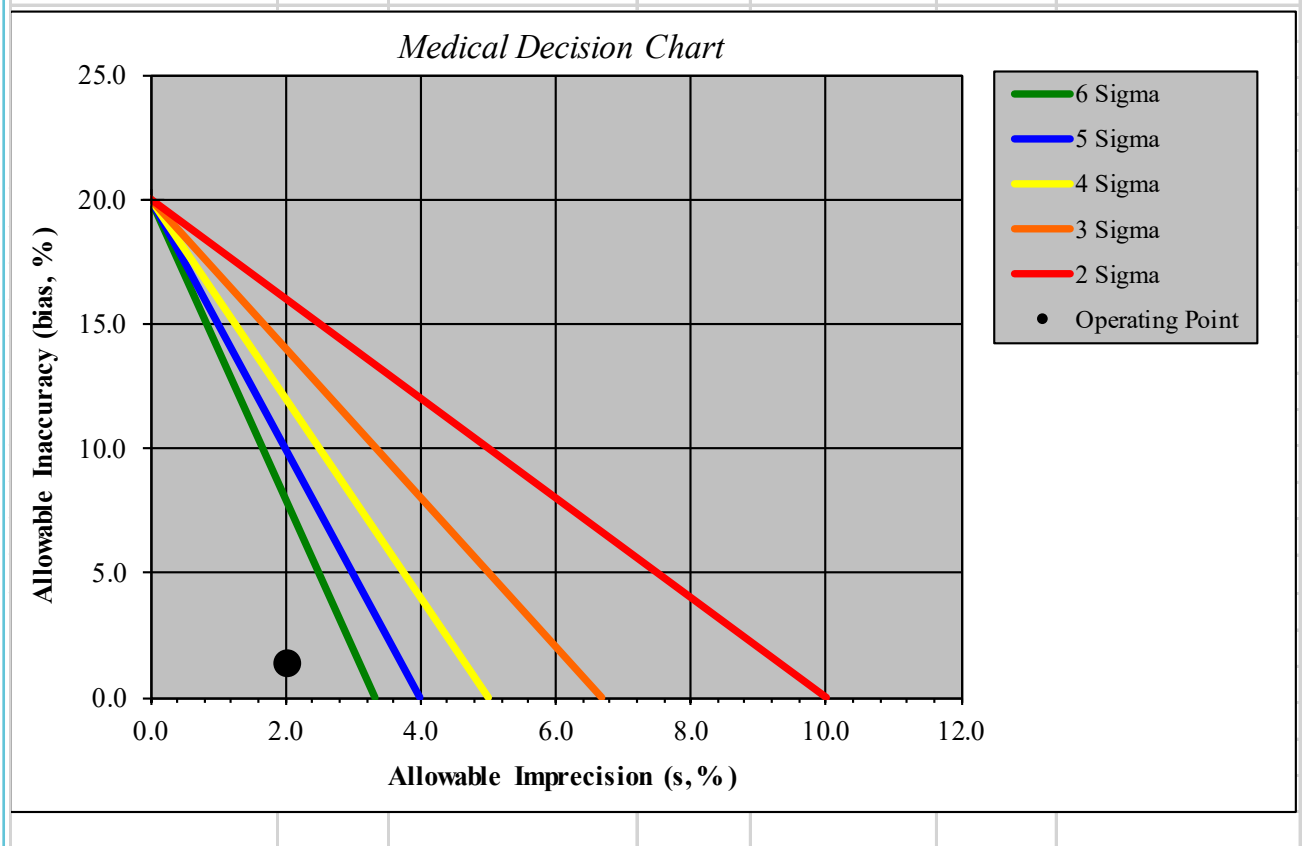


Chart 7 Method decision chart of L1, 33920 (Laboratory 2)

Test or Analyte	HbA 1c,33920,L1,Laboratory 2	Analyst	Dr.Shyamali Pal
Methodology	Ion Exchange HPLC	Date	
Quality Requirement		Sigma Limits	
Allowable Total Error	20.0	6	3.33
Offset	0.0	5	4.00
Method Performance		4	5.00
Bias (% diff)	2.4	3	6.67
Imprecision (% CV)	2.2	2	10.00
Sigma Metric	8.0		

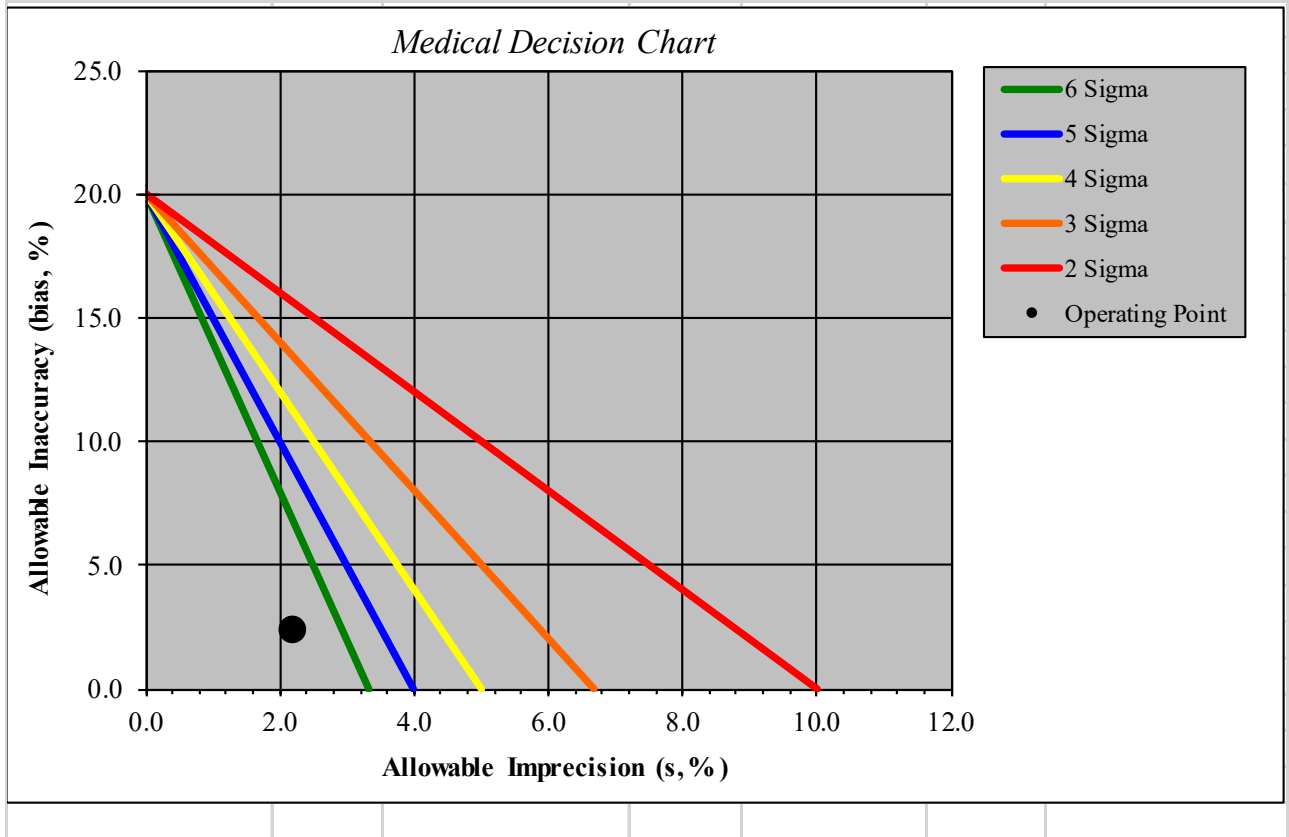


Chart 8 Method decision chart of L2, 33920 (Laboratory 2)

Test or Analyte	HbA 1c,33920,L2,Laboratory 2	Analyst	Dr.Shyamali Pal
Methodology	Ion Exchange HPLC	Date	
Quality Requirement		Sigma Limits	
Allowable Total Error	20.0	6	3.33
Offset	0.0	5	4.00
Method Performance		4	5.00
Bias (% diff)	3.5	3	6.67
Imprecision (% CV)	2.0	2	10.00
Sigma Metric	8.2		

